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Conference Report

Seventh International Workshop on the Fragile X and X-Linked Mental Retardation

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INTRODUCTION

The Seventh International Workshop on the Fragile X and X-linked Mental Retardation was held at the University of Tromsø in Norway on August 2–5, 1995 (Fig. 1).

Approximately 120 participants from 20 countries attended the Workshop. By special invitation Dr. Felix de la Cruz, who initiated the first international Workshop on fragile X, attended this Workshop. For the first time, the workshop took place in Scandinavia and was hosted by Lisbeth Tranebjærg and Herbert Lubs. For most participants this Workshop, held at the northernmost university in the world, presented a unique opportunity to visit this exotic place. Between sessions, the participants had a chance to experience 24 hours of daylight, codfishing, and extreme weather situations with excessive amounts of rain as well as spectacular changes in the light and rainbows. The format of the Workshop was a combination of platform presentations and poster presentations. In contrast to previous meetings, the Workshop opened with syndromal and non-syndromal X-linked mental retardation in order to allow time for discussion.

The first session was on X-chromosome inactivation. Huntington Willard gave a comprehensive update on X-chromosome inactivation and presented a new method [Carrel and Willard, this issue] on X-inactivation determined by differential methylation at the fragile X locus, [Willard, this issue; Rupert et al., 1995]. The methods now present a possibility for explaining unusual aspects of X-chromosomal inactivation of relevance for the large number of X-linked mental retardation conditions. One example of a large family with skewed X-inactivation in obligate female carriers of a gene for a rare X-linked recessive condition with mental retardation, dystonia, and deafness in a Norwegian family was presented by Ørstavik [this issue].

During a session for late breaking presentations, the main topic was premature ovarian failure in premutation female carriers of FRAXA [Conway et al., 1995; Turner et al., 1994]. Convincing evidence was presented pointing to increased risk of menopause before the age of 40 years in female premutation carriers of FRAXA. Previous studies [Schwartz et al., 1994; Vianna-Morgante et al., this issue] suggested this, which is now further substantiated. Howard-Peebles (Fairfax, VA) also reported that in IVF cycles, eight of nine premutation carriers had diminished response to ovarian stimulation, while four of nine had premenopausal FSH levels in apparent contrast with the fact that these women were fertile under normal conditions.

PROGRESS TOWARDS CLONING XLMR GENES IN PATIENTS

H. Willard and C. Schwartz

The localisation of X-linked mental retardation (XLMR) syndromes has increased tremendously since the 6th International Workshop. Much of the progress has been due to the utilisation of informative mi-

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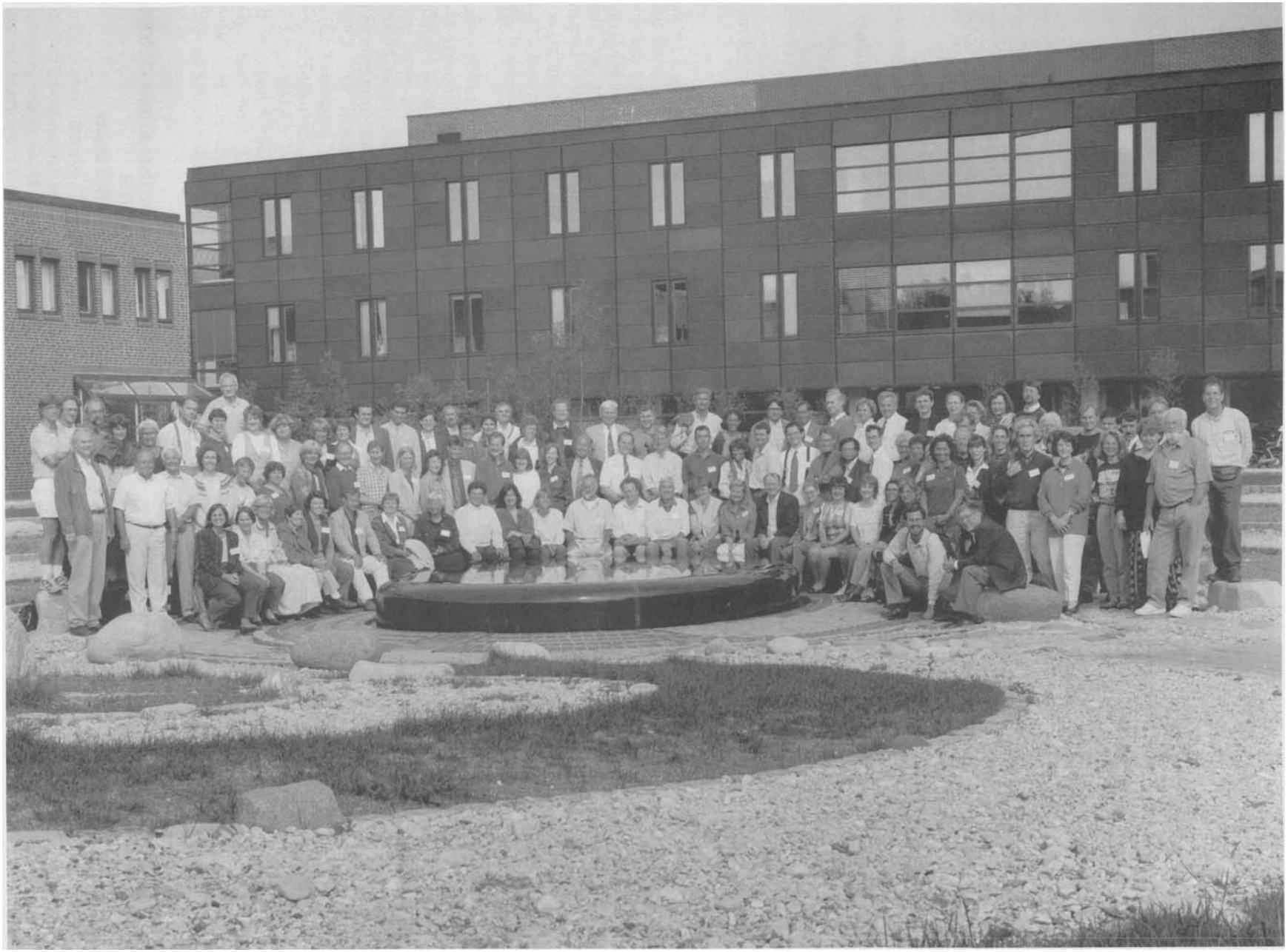

Fig. 1. Conference participants. Seventh International Workshop on the Fragile X and X-linked Mental Retardation, Tromso, Norway.

crosatellite markers that increased the potential for establishing linkage in families barely large enough to provide the prerequisite of seven informative meioses needed to exceed a lod score of 2. This has been particularly true for the group of entities referred to as nonspecific or non-syndromic XLMR. Whereas syndromic entities present a phenotype which makes them amenable to testing of "candidate genes" based on tissue expression of those genes, nonsyndromic entities could result from a defect in any gene with some expression in the brain. Recognizing this development, a session of the 7th International Workshop was devoted to methods used by various researchers to define discrete regions of the X chromosome which appear to be involved in brain development.

A. Smits (Nijmegen, Netherlands) presented work from the group of Ropers (Nijmegen, Netherlands) on their utilisation of alterations of the X-chromosome in patients in which mental retardation is the most salient abnormality. They have used three patients with inversions of the X and three patients with X-autosome translocations to define specific breakpoints that disrupt genes apparently involved in mental retardation. The inversions identified breakpoints in Xp11 and q21; Xp21 and Xq24; and Xq21 and Xq24. They are in the process of identifying cloned genomic material which crosses these regions. The translocations involved p11 (two translocations) and q13. For the latter translocation, a candidate gene has been identified in which the breakpoint is in the 5′ untranslated region of the 1st exon, about 1.5 kb from the start codon. The gene contains 26 exons and has an open reading frame consistent with a protein of 4,164 amino acids. However, to date Ropers and his colleagues have been unable to determine a mutation in any patients in their collection of XLMR families which map to the Xq13 region.

S. Briault (Tours, France) presented a similar approach employed by the group of Moraine (Tours, France) to map two potential locations for the FG syndrome. They studied a family in which an inversion of the X chromosome, inv(X)(q11q28) segregated with the phenotype. Physical mapping of the breakpoint places the q11 locus in a YAC positive for DXS1 and the q28 locus in a YAC positive for DXS18. A search for the candidate gene at each location is in progress.

The laboratory of Ropers has utilised the existence of males with small deletions of proximal Xq21 and MR (± chorioderemia or deafness) to define a region of about 300 kb, which should contain a gene involved in XLMR. The limits of the region are defined by the markers DXS995 (proximal) and DXS232 (distal), primarily based on two patients, AP and LGL2905 [Van der Maarel et al., 1995]. Using a similar approach, L. Colleaux (Marseilles, France) presented work from the groups of Fontes (Marseilles, France) and Schwartz (Greenwood, SC), which would appear to localise a MR gene between DXS233 (proximal) and CHM (distal), a region of about 1.2 Mb [May et al., 1995]. This work was based on the analysis of a patient (TM) with chorioderemia and mental retardation and would appear to be at odds with the data presented by Smits. Further research by both groups will likely answer this question.

As had been noted by Schwartz and his colleagues, the proximal long arm of the X chromosome has a cluster of genes associated with syndromic and nonsyndromic XLMR entities. Recognizing the importance of this, the laboratory of Fontes has undertaken construction of a physical and transcription map of the region Xq11-q21.3. M. Fontes presented their current progress in reaching this goal. Utilising three different YAC libraries they have been able to cover an estimated 18 Mb, representing about 80% of this region. Direct cDNA selection has been performed using these cloned resources resulting in the identification of numerous new genes. Based on their tissue specificity, many are candidates for *XLMR* conditions. In fact, mutations in one of these genes, XNP, was shown to be associated with ATRX (alpha-thalassemia, mental retardation, X-linked) [Villard et al., 1996a,b; Gibbons et al., 1995].

M. Schmidt [Melbourne, Australia, this issue] presented a deletion map of the Xq27→q28 region, which should assist in searching for mental retardation syndromes located in this gene-rich region of the X chromosome. Using molecular analysis of markers absent in patients with deletions without a specific syndromic phenotype, it was possible to exclude that respective syndromic gene from the area of the deletion. This allowed the narrowing of regions of localisation for the following syndromic *XLMR* conditions: *BFLS,* WSN, and MRSD.

X-LINKED MENTAL RETARDATION SYNDROMES: POSSIBLE NEW SYNDROMES

Jean-Pierre Fryns

In the first part of a session on syndrome forms of XLMR two possible new XLMR syndromes were presented.

Dr. J. Leisti (Oulu, Finland) presented a family with a form of XLMR: in this family six affected males in three generations presented progressive early-onset mental retardation, epileptic seizures, asymmetry of skull, prominent auricles, flat nasal bridge, and hypertelorism. No abnormalities were noted in carrier females. Dr. Leisti's interesting family illustrates very well the difficulties in delineation of new syndromic forms of XLMR: although the pedigree data are most compatible with X-linked inheritance, the clinical and neurological findings in the affected males are not very distinct and make clinical recognition in an individual male patient without a family history of XLMR extremely difficult. Moreover, as in many other families with severe forms of XLMR combining neurological and clinical symptoms and leading to early death in affected individuals, DNA from affected males in previous generations is not available for linkage studies.

The Nijmegen group (Dr. Hamel) presented a family with XLMR and isolated growth hormone deficiency. No other anomalies were present in affected males in this family. Moreover, the degree of mental retardation was variable and ranged from borderline/dull to severe. Molecular analysis located the gene to Xq24-27.3.

Finally, Dr. Telvi (Paris, France) presented a poster on a French family with XY gonadal dysgenesis, multiple congenital abnormalities, and mental retardation in affected males belonging to three generations. Duplication of the Xp21.3 region was excluded. Data were compatible with X-linked recessive or autosomal dominant, sex-limited transmission.

NEW STUDIES IN KNOWN XLMR SYNDROMES
Patrick Willems

Michel Fontes (Marseille, France) reported patients with XLMR and minor anomalies reminiscent of Coffin-Lowry syndrome.

Although these patients did not show any sign of α-thalassemia, they had mutations in XNP, a potential transcription factor involved in ATRX (X-linked α-thalassemia-retardation syndrome). This suggests that XNP might be implicated in XLMR more frequently than previously thought.

Patrick Willems (Antwerp, Belgium) reviewed the genetics and clinical spectrum of X-linked hydrocephalus (HSAS), MASA syndrome, X-linked complicated spastic paraparesis (SP1), and X-linked corpus callosum agenesis (ACC).

These four conditions have an overlapping phenotype and are all due to mutations within the gene L1CAM which is located in Xq28 and encodes for a neuronal cell adhesion molecule involved in the development of the brain. Nearly all L1 mutations are private mutations dispersed over L1 without any evident genotype-phenotype correlation.

John Mulley (Adelaide, Australia) reported on a refined localisation of three different XLMR syndromes. The gene responsible for XLMR with spastic diplegia and microcephaly (MIM 309470) is located in a 22 cM pericentromeric region between MAOA and DXS1125/DXS106. The Börjeson-Forssman-Lehmann syndrome (MIM 301900) gene is now located in Xq26-q27 in a 25 cM interval distal of DXS425. The localisation of the gene responsible for XLMR with gynecomastia and obesity (MIM 309585) was refined to an interval of 25 cM in Xq21.3 between DXS426 and DXS990.

NON-SPECIFIC XLMR
Herbert A. Lubs

During this session six presentations covered 11 new MRX families. Most of these reports can be found in detail in the present issue in papers from the groups in Leuven, Adelaide, Miami, and Greenwood. Other presentations were made by groups from Germany and Israel. An excellent compilation and review was provided by Mulley (see next session) and in the related paper in the present issue by Gedeon et al. entitled "How many X-linked genes for nonspecific mental retardation are there?"

The most exciting paper was presented by Raeymaekers, who outlined the route through localisation by multipoint linkage studies in a family with six affected males (subsequently numbered MRX34) to saturation linkage studies in this region, to the ultimate finding that DXS1218 (located in Xp21.3) was deleted in all affected males. To date, no MRX gene has been cloned. This study will likely lead to the first cloning of an MRX gene, since the deletion was less than 1 Mb, and will serve as a prototype for an important alternative approach for cloning these disorders. Over 40 families were known at the time of the meeting, and a total of 41 MRX assignments have now been made.

OVERVIEW OF ONGOING XLMR STUDIES
John Mulley

This session included a number of reviews to complete that section of the Workshop devoted to non FRAXA/FRAXE X-linked MR.

Ben Hamel summarised the XLMR families studied at Nijmegen. One had syndromal signs similar to those described in families reported by Vasquez et al. [1979] and Wilson et al. [1991], with a localisation similar to that reported earlier in the day for the Wilson et al. [1991] family. Another, with non-specific XLMR, had a PLP duplication which was of interest given the clinical diversity already known at this locus (spastic paraplegia 2 and Pelizaeus-Merzbacher disease). Surprising absence of linkage to markers on the X chromosome was noted for one family with an apparent X-linked inheritance pattern. This reminded us of the pitfalls in linkage studies such as undetected non-paternity in early generations of a large pedigree, phenocopies and difficulty in the classification of borderline XLMR.

Claude Moraine (Marseille, France) described a series of French families and expanded on the theme that well documented clinical and neuropsychological studies were an essential foundation for mapping. Difficulties were encountered in distinguishing between syndromal or nonspecific XLMR in some families. Any associated physical phenotype needed to be defined by comparison between affected men and their unaffected relatives. This could be complicated by variable expressivity within families, especially where MR was borderline. Congenital hypotonia and refractory seizures were frequently observed. Again, there appeared to be an over-representation of XLMR genes in the Xp2l-Xq2l region of the chromosome.

The first of two presentations from Herb Lubs (Miami, FL) continued to emphasise the characterisation and mapping of XLMR families. The Miami/Greenwood study now incorporates 62 families and has played a major role in the refinements to both clinical definition and mapping. Such studies encompassing large numbers of families lead to a proportion amenable to molecular characterisation of XLMR genes. However, positional cloning in the absence of structural chromosomal abnormalities remains impractical and represents the major barrier to the characterisation of the XLMR genes responsible for the large numbers of families now being characterised and mapped.

Finally, Herb Lubs presented the traditional update on monogenic XLMR disorders (reviewed in previous years by John Opitz and Giovanni Neri). Although there had been no new localisation for syndromal XLMRs, spectacular progress in the mapping of MRX

(nonspecific XLMR) had led to an increase from 19 localisations at the previous Workshop to approximately 50 localisations at the present Workshop. The existence of at least eight nonoverlapping regions spread along the chromosome from Xpter to Xqter suggests a minimum number of eight MRX genes. The prerequisite for publication of an MRX localisation is assignment of an MRX number from Phyllis McAlpine, Nomenclature Editor, Genome Database (Fax: +1-204 786 8712, Email: mcal@genmap.Lgen.umanitoba.ca). This requires a lod score of ≥2 (as the agreed significance level for the linkage to the X chromosome) together with the nearest flanking markers which define the regional localization. The highlight of the XLMR update was the recognition of defects in the XNP gene as responsible for the syndrome of a-thalassemia with MR (ATR-X) [Gibbons et al., 1995]. Defects within the same gene are now known to cause Juberg-Marsidi syndrome [Villard et al., 1996 a] and a syndromeal form of XLMR without alpha thalassemia [Villard et al., 1996b].

As the day concluded the importance of international collaboration was emphasised to bring together the clinicians with families and the molecular geneticists with the candidate genes or the technology to identify new genes. Virtually everything about the molecular bases for these heterogeneous XLMRs remains to be discovered, by comparison with fragile X syndrome where progress during the first half of this decade has been spectacular.

POPULATION SCREENING
Patricia Jacobs

Six papers were presented in the session on population screening. In the first of these Gillian Turner (Newcastle, Australia) presented data on the prevalence of the FRAXA full mutations based on the retesting of individuals found to be fragile(X) positive on cytogenetic screening in the New South Wales and Coventry population surveys. In both populations retesting the fragile X individuals showed a number with no molecular evidence for either a pre- or full mutation. Recalculation of the prevalence of the fragile X full mutation based on these more accurate figures gave a prevalence of affected males of approximately 1 in 4,000 to 1 in 5,000, with a corresponding figure of affected females of half this [Turner, this issue]. Data have recently been presented by Rousseau et al. [1995] indicating that 1/259 females are premutation carriers. Pat Jacobs (Salisbury, UK) then presented preliminary data on molecular screening of a large population of boys with special educational needs and their mothers for mutations at either the FRAXA or FRAXE loci. The result on approximately 700 boys and 500 mothers were reported: only four full FRAXA and zero full FRAXE mutations and a single FRAXE premutaton were seen. These data confirm that the frequency of FRAXA full mutations is considerably less than previously thought and suggest that FRAXE full mutations are rare.

Jean-Louis Mandel (Strasbourg, France) presented data from 3 years of molecular diagnosis of the Fragile(X) syndrome in France suggesting that this condition is underdiagnosed in that country, the recent data showing an average age of diagnosis of 16 years. Patricia Howard-Peebles (Fairfax, VA) presented data on fragile X testing in routine prenatal clinics and on the testing of potential donors in an anonymous egg donor programme. Among 418 pregnant females with a negative family history with respect to mental retardation three premutations were detected with repeat numbers of 60, 64, and 67, while among 237 egg donors 2 women were found with repeat numbers over 50 (52 and 54, respectively).

Ted Brown (New York, NY) reported on screening, using a PCR protocol that recognises normal alleles and pre- and full mutations, of pregnant women with a family history of mental retardation Among 320 women tested he reported one full and one premutation and two unstable alleles of sizes 59 and 56, respectively. He also presented data that showed the PCR technique to be suitable for use as a prenatal test and as a test for detecting fragile X in males with developmental delay of unknown origin. Unfortunately Dr. Falik-Borenstein could not be present and preliminary data on haplotypes suggesting that there was a founder effect that might contribute to the apparently high frequency of fragile X among Tunisian Jews was presented by James Macpherson (Salisbury, UK).

FOUNDER EFFECT AND METHODOLOGY
Stephanie Sherman

This session involved two different areas of study of the fragile X syndrome: 1) studies of the association of the fragile X mutation and flanking markers and 2) assessment and development of diagnostic methods of the fragile X mutation. With respect to the first area of study, it was found that specific polymorphic markers (primarily dinucleotide repeat markers) that flank the CGG repeat region of the FMR1 gene are associated with the fragile X mutation; more recently, this association has been found to be extremely complex. Many interpretations of the observations have been suggested. It may be that the association is the result of 1) a founder effect, 2) a predisposition towards stability or instability on particular haplotype backgrounds, 3) a consequence of the FMR1 mutation itself leading to unique flanking markers, 4) a gene promoting instability of this entire region, or 5) to a combination of these possibilities. The reports presented at this meeting provided evidence for several of these possibilities, but none were conclusive. Leisti et al. (Oulu, Finland) presented data on the population from the north of Finland. One particular haplotype was found in 80% of the fragile X chromosomes and in only 8% of normal X-chromosomes. This is the strongest association found to date. This association is clearly the result of a founder effect. This conclusion is further substantiated by the church records, starting from the 16th and 17th century.

Brown et al. (New York, NY) also analysed a second population from Finland and again found a strong association. They reported that there were four rarer haplotypes in addition to the one ancestral type that predominated. One interpretation is that other inde-

pendent new mutations have occurred in this same population. This observation is one that has been noted in the other more admixed population.

Macpherson et al. (Salisbury, UK) provided a summary of the characteristics of the FMR1 association that they found through their screening surveys in Britain. The observations are based on the frequencies of haplotypes in three parts of the population: 1) among those with normal CGG repeat sizes (less than 35), 2) those with an intermediate range (35–50), and 3) those with pre- or full mutations. Three general observations were reported. They found that several haplotypes were rare or absent in the normal and intermediate population, but more common among those with the fragile X mutation. Other haplotypes that were observed in the normal population (most often among those with repeat sizes in the low 30s) were at a higher frequency in the fragile X mutation population. Last, there was one specific haplotype that was overrepresented in the intermediate population. Each of these observations suggests a different mechanism that may lead to an association.

Recently, Eichler et al. [1996] further characterised one CGG-AGG pattern in this same British population and found evidence for at least two mutational pathways: 1) one due to frequent recurrent loss of the 3′AGG leading to a rapid progression towards the premutation state and 2) one resistant to loss of the 3′AGG leading to a slow progression towards the premutation state.

Brown et al. (New York, NY) also provided evidence that there was a correlation between CGG repeat size and microsatellite dinucleotide repeat size. They recently suggested a molecular mechanism to explain this observation [Brown et al., 1996]. Further studies are needed to identify specific mechanisms. Macpherson et al. (Salisbury, UK) suggested that further investigations may involve 1) identification of families with other repeat sequence disorders to search for possible genes that cause general genomic instability, 2) correlation studies between AGG interspersion patterns with haplotypes and 3) examination of haplotype associations in other ethnic groups as well as done by Kunst et al. [1996].

Investigations of associations in other populations have begun and several were reported in this meeting. They include a study of an Hellenic population [Syrrou et al., this issue], of an Italian population [Chiurazzi et al., this issue] and of a sub-Saharan population [Chiurazzi et al., this issue]. Comparison of these particular studies as well as those in the literature is not straightforward because different flanking markers are used, nomenclature of alleles is not standard, and PCR techniques vary [Chiurrazzi et al., this issue]. It was suggested that one large family, perhaps one of the CEPH families, be analysed by several laboratories to determine the characteristics of the FMR1 CGG repeat region, including AGG interspersion pattern and flanking markers. The resulting genotype information using a standard nomenclature as well as the DNA for the individuals in the family would then be available to the scientific community. This standardization would be invaluable to ensure that all studies are comparable.

With respect to the second area of study presented in this session, diagnostic methods to identify the fragile X mutations, Dr. Oostra (Rotterdam, Netherlands) and his colleagues presented an exciting new development of an antibody test [Willemsen et al., 1995]. This test provides a method for rapid screening of the fragile X syndrome. It requires examination of lymphocytes from freshly made blood smears and would only take a few minutes per sample. Using this antibody test, males with either no or very low expression of the FMRP protein and possibly females with full mutations could be identified.

Although this type of test will be ideal for detection of those individuals with altered levels of FMRP (protein), it will still be necessary to use other methods to detect the repeat number in the FMR1 gene to assess the premutation carrier status. Currently, Southern blotting and PCR techniques are used for this assessment. Kajanoja et al. (Kuopio, Finland) described the diagnostic advantages and disadvantages of linkage analysis, Southern blotting and PCR methods based on the assessment of a large four generation family with the fragile X syndrome.

Although the molecular techniques are quite standard, different approaches can cause variability in test results. Fisch et al. (New York, NY) investigated a problem that may be encountered in any diagnostic test: inter-rater reliability of the interpretation of test results. They examined the reliability among diagnosticians in different laboratories reading the same set of autoradiographs of Southern blot analysis of the fragile X mutation. They found that, in general, interpretations were similar.

Two groups developed new non-radioactive PCR methods for identification of individuals with the fragile X syndrome [Haddad et al., this issue; Pascale et al., this issue]. These new approaches are less expensive and more rapid than those currently available and, thus, are well suited for clinical settings and, possibly, for large population screening.

In summary, assessment and development of methods used to determine the CGG repeat number for carrier detection and to identify altered levels of protein production for detection of individuals with the fragile X syndrome were presented. New advancements indicate that rapid, inexpensive and reliable tests will be available for clinical and research settings.

FRAXA MOLECULAR STUDIES AND STUDIES OF TRANSMISSION REPEATS

Jean-Louis Mandel and Ben Oostra

Willems et al. (Antwerp, Belgium) presented the FMR1 knockout transgenic mouse model [Kooy et al., this issue; Bakker et al., 1994]. Their mice had now been followed for a longer period of time than in the first presentation [Bakker et al., 1994] and after an initial, progressive macroorchidism this decreased when the mice were followed up to 170 days. No histological abnormalities could be documented. In the observations of cognitive function the mutant mice showed slower learning capability even if the differences were small. The visuo-spatial disabilities of the knockout

mice were also investigated by Godfraind et al. (Antwerp, Belgium) but no impaired, long-term potentiation (LTP) in the hippocampal area could be demonstrated. It will remain to be investigated whether the demonstration of increased volume of the caudate nucleus in human brains demonstrated by Reiss et al. [1995a] can be demonstrated in the mouse model.

While it is now well accepted that FMR1 codes for a set of RNA binding proteins (the multiplicity of FMRP isoforms observed in vivo resulting from alternative splicing), their function is still a mystery, Jean-Louis Mandel (Strasbourg, France) reported that isoforms lacking exon 14, that are predicted by the alternative splicing pattern (and which would have a different carboxy terminus that the major isoforms), appear localised in the nucleus when expressed in cultured cells, following transfection with appropriate expression vectors. Further experiments showed that sequences coded by exons 1 to 8 may contain a nuclear association domain, while exon 14 encoded sequences are indeed necessary for the cytoplasmic localisation of FMRP and may correspond to a cytoplasmic retention signal, or to a nuclear export signal [Sittler et al., 1996]. The latter signal was recently described in proteins that shuttle between the nucleus and cytoplasm, and that includes some RNA binding proteins [Moore, 1996]. Important new findings by the group of Gideon Dreyfuss (Philadelphia) were reviewed by Mandel. Siomi et al. [1995] and Zhang et al. [1995] have reported the existence of two autosomal genes highly homologous to FMR1, which they called FXR1 and FXR2. The corresponding proteins have also RNA binding properties and are able to form homodimers, or heterodimers with FMRP. The expression pattern of FXR1 was studied by Coy et al. [1995] in mice and appears quite different from that of FMR1 (no expression in differentiated neurons, high expression in skeletal muscle, postmeiotic spermatids and in actively proliferating layers in the brain). The recent finding by Khandjian et al. [1996] that FRMP is associated with ribosomes (notably the 60S subunit) adds a new complexity to the problem of FMRP function [Khandjian et al., 1996]. Does FMRP bind both to some mRNAs (as suggested by Ashley et al., 1993) and to ribosomal RNAs present in the 60S subunit? Is it a translation regulation factor, or is it involved in ribosomal assembly?

The relationship between methylation, FMR1 expression and phenotype were discussed in several presentations. Ben Oostra reported that in a patient with a full mutation carrying a lung tumour, a premutation size (160 repeats) was found in the tumour that unexpectedly was methylated at the EagI and BssHII sites usually tested, while a high level of FMR protein was detected histochemically. This is the first case of apparent dissociation between methylation and absence of FMR1 expression, suggesting that methylation at the sites analysed may not be critical for expression. Analysis of other tissues from the same individual showed that FMRP staining may pick mutation mosaicism undetected by Southern blot analysis, but confirmed by PCR. Another interesting case study was presented by Arie Smits: two intellectually normal brothers (one of the grand father of a mentally retarded boy with a full mutation) were found with 13 and 7% fragile site expression, respectively, and presence of a large (mutation size) unmethylated expansion. FMRP was detected immunochemically in a blood smear test, albeit at perhaps reduced levels. This is in apparent contradiction with the report by Feng et al. [1995] that in the absence of methylation, repeats of more that 250 CGGs inhibit translation of FMR1 mRNA almost completely. Patricia Howard-Peebles (present issue) has studied other patients with apparent discrepancies between phenotype and genotype, by comparing mutation pattern in leukocytes and epithelial buccal cells. One mentally retarded boy with a small premutation of 60 repeats in blood was thus found to be mosaic for this premutation and a methylated full mutation in cheek cells.

On a more fundamental vein, Peter Steinbach [present issue] proposed that methylation of the CGG repeat might stabilise it, accounting for the difference with the greater somatic instability of the unmethylated CTG repeat in myotonic dystrophy, seen in cultured fibroblasts. He hypothesised that methylation directed mismatch repair might be involved. The parental bias in transition from premutation to full mutation could then be explained by a paternal allele specific methylation established during spermatogenesis, while absence of this methylation on the maternal allele at the preimplantation stage would cause abnormal repair and instability, followed by de novo methylation and stabilisation.

A study of AGG interspersion and instability as reported by N. Zhong and S. Nolin showed that, while a majority of normal alleles have two AGGs, one third of premutations had only one AGG, while the remaining had no AGG. For the grey zone alleles, those with two or three AGGs are most likely stable; in their series, instability was observed above a threshold of 41 pure CGGs, while stable transmission in two meioses of an allele with 50 pure CGGs, and finding of sibship clustering in the pattern of instability led to the suggestion that other familial factors may modulate instability. Analysing transmission from fathers with premutations to their daughters, Nolin suggested that in the 56–80 repeats range, there are more expansions than no change or contraction, while in the 80–100 range, expansions were as frequent as contractions. The session ended with presentation of an elaborate population genetics model, with expansion assumed to occur during both meiosis and early embryogenesis. This model [Ashley and Sherman, 1995] predicts that the risk to expand to full mutation does not depend only on the observed size of the premutation, but also on its parental origin. It also predicts that some males with full mutations should have also a full mutation in sperm.

Holden et al. (Kingston, Canada) presented a rapid method for study of numerous di- and tri-nucleotide repeat markers including FRAXA, FRAXE, and FRAXF on dried bloodspots from Guthrie-cards of newborn infants. The method will be important for newborn screening programs for FRAXA and FRAXE.

FRAGILE X: BEHAVIOR AND BEHAVIOR/MOLECULAR CORRELATIONS

Gene Fisch and M. Borghgraef

The session began with presentations concerning heterozygous females with the FMR-1 mutation. Bert deVries (Rotterdam, Netherlands) examined their mental status using first degree relatives as controls. On average, females with full mutations had significantly lower IQ scores, nearly half of which were below 70. In addition, their activation ratios (AR), the proportion of active normal X chromosome to total active chromosomes, were moderately correlated with IQ scores. These findings are in accordance with results obtained previously by Abrams et al. [1994] in which IQ scores of heterozygous carriers were moderately correlated with AR.

Petra Franke (Mainz, Germany) examined heterozygous carriers of normal intelligence and compared them with other, unrelated normal controls to determine whether the presence of psychopathology was greater among fra(X) females. In most respects, fra(X) females differed little from controls but the proportion of overall psychopathology was higher among fra(X) females. The prevalence of psychopathology was also greater in families of fra(X) females. As these researchers state, their findings are controversial since previous studies found no difference in the prevalence of psychopathology among fra(X) and non-fra(X) females with normal IQ scores.

Martine Borghgraef (Leuven, Belgium) examined the neuropsychological, psychopathological, and cognitive characteristics in two small groups of heterozygous females. In adult female carriers with normal IQ scores, all but one exhibited higher performance than verbal skills. In young females with full mutations, mean IQ scores were in the low normal range but they showed better verbal than performance skills. Both groups manifested deficits in visual memory and attention. Extensive psychiatric evaluation could not confirm a high prevalence of behavioral problems found by Franke et al. [this issue]. Moreover, there was no correlation between IQ and mutation size.

The session on females concluded with a presentation by Gail Spiridigliozzi (Durham, NC), who reported four case studies of premutation carriers. Their ages ranged from toddler to adult. Cognitive abilities ranged from mild MR to above average IQ with learning disabilities. According to this investigator, these females appear to have abnormalities similar to but milder than females with the full mutation.

Behavioral studies of fra(X) males were presented in the second part of the session. Gene Fisch (New York, NY) presented data from their 4-year prospective multicenter study in which cognitive skills (IQ) and adaptive behaviour levels (DQ) of young, fully mutated fra(X) males were tested and retested. Using one test for IQ and a second for DQ, they found that both scores declined for most males; and that adaptive behavior was superior to cognitive ability in more than 90% of those tested. These findings confirmed previous reports of retrospective longitudinal declines in IQ. Decreases in IQ and DQ were unrelated to the size of the fra(X) mutation.

Randi Hagerman (Denver, CO) presented data from her retrospective study of fra(X) males and females, which corroborated the results of the prospective study of males by Fisch. They noted that both fra(X) and control subjects exhibited declines in IQ scores. However, fra(X) males manifested significantly greater declines than controls, while fra(X) females do not show greater decreases than controls.

Fisch presented results from a study in which the correlation of cognitive and behaviour deficits with mutation size were calculated. They found that neither IQ nor DQ were associated with mutation size. Hagerman also noted that cognitive deficits were unrelated to mutation size in samples of fra(X) males and females, confirming the findings obtained by Fisch et al. [this issue] and Borghgraef et al. [this issue]. However, among females, AR (activation ratio) was correlated with degree of cognitive deficit and physical findings, corroborating the results obtained by de Vries et al. [this issue].

In a study of young mosaic males, Sarah Nolin (New York, NY) presented findings from their study of autistic and non-autistic fra(X) subjects. Unlike the results obtained by Rousseau et al. [1994], these researchers noted that mosaic males had a higher level of development of adaptive behaviour than those with the full mutation.

Jean Steyaert (Leuven, Belgium) presented the last paper of the session. He reported on cognitive and clinical findings in seven fra(X) males with mutations from the "grey zone" size between 170–300 repeats. While there was no correlation between mutation size and degree of methylation, there was a strong correlation between IQ score and degree of methylation. These findings support the results obtained by de Graaff et al. [this issue] and suggest further that some protein (FMRP) is made when the mutation is partially methylated.

Recently, Reiss et al. [1995a] presented data indicating that FMR1 full mutation individuals (males and females) have increased volume of the caudate nucleus and, in males, lateral ventricles. Quantitative neuroimaging studies will become an additional tool to establish genotype-phenotype correlation. In a very elegant study of FRAXA full mutation females [Reiss et al., 1995b] the authors showed that the activation ratio is a strong predictor of measure for which persons with fra(X) show greatest impairment, compared to relatively spared cognitive abilities.

Presentations from this session can be summarised broadly as follows: 1) but for size differences between premutations and full mutations, the number of CGG repeats in the fra(X) mutation does not appear to be responsible for the clinical, cognitive, behavioural, or psychopathological traits of the fra(X) syndrome; and 2) the extent to which the fra(X) mutation is methylated affects the amount of mRNA transcribed and, in turn, the quantity of FMRP produced. Variable amounts of FMRP are associated with the degree to which the clinical, cognitive, behaviour or psychopathological abnormalities will be manifested.

FRAGILE X COUNSELING AND CLINICAL STUDIES

Randi Hagerman

The session opened with a presentation by Puissan (France) regarding fragile X syndrome and the FG syndrome-like phenotype. He presented a family with six children affected by fragile X syndrome; however, in four of the brothers FG syndrome was the preliminary diagnosis because of the presence of mental retardation, seizures, frontal bossing, cowlick, macrocephaly, hypotonia, feeding problems, constipation, hyperactivity, everted lower lip, and thick philtrum. Puissan emphasised that the FG syndrome may overlap with other XLMR disorders including fragile X syndrome, spasticity, and contractures were not present. Loesch et al. [1992] previously reported an association between facial and digital abnormalities and fragile X syndrome, including one case of apparent FG syndrome.

Staley-Gane (Denver, CO) subsequently discussed pertinent issues regarding genetic counseling in families with fragile X syndrome. Because of the spectrum of emotional, physical, and cognitive problems associated with fragile X syndrome the families often have a variety of concerns that they present during the genetic counseling session. Staley-Gane presented a new method, using the Q sort, which provides parents an opportunity to prioritise their needs and concerns prior to the genetic counseling session. The sorting of 16 items on a spectrum of most important to least important takes approximately 10–15 minutes and is a convenient format that can be utilised in any center which provides genetic counseling. Preliminary data from 27 families who took the Q Sortin Denver demonstrate different needs and concerns for fathers versus mothers, and those families with young children compared to those families with older children. The Q sort provides a guide to more carefully meet the needs of families with fragile X or other genetic disorders and to expand the role of the genetic counselor to work with psychosocial needs and concerns of families.

McConkie-Rosell (Durham, NC) presented data on the attitudes regarding carrier testing in fragile X syndrome which is part of a 3 year study involving a structured interview of carriers. The attitudes of 28 female carriers were reported here. Generally, the carriers felt that prior knowledge of their carrier status would have changed their reproductive plans, with 82% stating that they would have used prenatal diagnosis. All felt that sib and relatives should be told of their genetic risk, and 75% felt relatives should be told by relatives. All wished they had learned the information earlier and 60% said it would have been advantageous to have known their carrier status by middle school. In this study the female carriers felt that their children should be informed about the testing by 12 years of age and that the optimal time for testing was 8 to 10 years. The carriers felt that the information should be introduced gradually and in an age-appropriate manner. Although only three females had the full mutation, 32% of the carriers felt that they had emotional or learning problems which were the result of being a carrier.

Staley-Gane [Wingrove et al., present issue] presented a study regarding the risk of genetic discrimination in Colorado families with fragile X syndrome. Thirty-nine families participated. No families were denied insurance after genetic testing demonstrated fragile X. However, six families were declined full family coverage when they applied for insurance because of an affected index-case and two families were given riders which excluded health expenses involving fragile X. As part of the underwriting process known health problems are often excluded from coverage and fragile X is typical in this regard. Although no instances of dropped coverage occurred because of genetic testing, 15% of families would not reveal testing results to insurance companies. Thirty-six percent of families were afraid to change jobs and sixty-six percent had moderate to extreme worry regarding genetic discrimination. In June of 1994 Colorado passed a law which prevents insurance companies from requesting or utilising the results of genetic testing, including cytogenetic or DNA testing in their determination of insurability. Although a few other states have similar laws, there is a great need for federal legislation to protect the privacy of genetic testing results.

Robinson (Sydney, Australia) presented on the impact of genetic counseling on the prevalence of fragile X syndrome. In the evaluation of 204 carriers from 195 families she found that 77% took up the option of antenatal testing. The number of pregnancies from 1991 onward decreased and the number of terminations increased related to genetic counseling. Incidence rates for fragile X syndrome in NSW Australia has decreased 10-fold from 4.3/10,000 to 0.5/10,000 because of their genetic counseling program.

FRAXE SECTION

W. Ted Brown

Jean-Louis Mandel (Strasbourg, France) described his laboratory use of an alternative probe for FRAXE. He advocated using this probe (OxE18) rather than the originally used probe (OxE20) as it offers several advantages. It can be used to detect FRAXE locus expansion using the same EcoRI blots that may have been used for FRAXA screenings. It is also sensitive to the methylation status of the CGG repeat region as it detects an adjacent EcoRI methylation sensitive site. The probe was used to screen a series of approximately 300 mentally retarded subjects. They detected one FRAXE family including a mother with an unmethylated premutation and seven children, six with large methylated full mutations and one with an unmethylated premutation. The six children had social problems and mild or moderate mental retardation. They also restudied two families previously reported as positive for a fragile site at Xq27 but negative for FRAXA mutations finding that both had FRAXE mutations and the three affected males had mild mental retardation. Combined cytogenetic and molecular FRAXE prenatal diagnosis detected one affected male fetus and this pregnancy was being continued. Summarising their findings, Mandel concluded that the expansion threshold for methylation appears to be approximately 130–150 repeats and thus

is smaller than FRAXA. On questioning, Mandel noted they had seen decreases in repeat sizes in FRAXE transmission from premutation fathers to daughters.

Nan Zhong (New York, NY) reported on a survey to determine whether a correlation exists for the size of the FMR1 repeats and FRAXE repeats in normal and fragile X chromosomes in three different populations including samples of Caucasian, Chinese, and Finnish. No overall size correlation was apparent. However, among 419 Caucasian X chromosomes surveyed, one had a 59 bp microdeletion adjacent to the FRAXE CCE sequence. In addition, it had a complex completely unmethylated "premutation mosaic" expansion of the FRM1 CCG repeat extending from 60 repeats to a typical full mutation size. The male was cytogenetically positive at Xq27.3 (22%). Both the FRAXE microdeletion and FRAXA mutation were transmitted to his FMR1 premutation daughter (~120 repeats), who had two affected, full FMR1 mutation children. This grandfather was considered to be non-retarded but "slow." It seemed likely that the FRAXE deletion had little or no effect on his phenotype. In related studies a 5-year-old boy with slow speech and hyperactivity, suspected for fragile X due to positive cytogenetic results, was found to be negative for a FMR1 mutation but was FRAXE positive with a large expansion and a faintly incomplete methylation pattern. His normal mother was a carrier with an expanded 8 kb band plus a normal 5.2 kb.

Pablo Carbonell (Murcia, Spain) described FRAXE mutational analysis of three Spanish families. The affected males tended to have a brachycephalic head shape with a narrow forehead and telecanthus. One affected male was noted to have macro-orchidism. The affected males were hyperactive and had highly variable deficits in mental status. A prenatal cytogenetic study had demonstrated Xq-terminal fragility and postnatal studies confirmed the presence of the FRAXE mutation.

Alessandra Murgia (Padova, Italy) reported on a FRAXE screening program in Italy. All FRAXA negative subjects are subsequently screened for FRAXE mutations. One of 150 subjects screened in this manner was identified with a FRAXE mutation. This 4-year-old boy was severely speech delayed and judged to be mentally retarded. He had hypoplasia of the midface, micrognatia, and bilateral clinodactyly. However, his "normal" 15-year-old brother had similar molecular findings of a expanded hypermethylated FRAXE locus although neither evidenced cytogenetic fragility.

Following the conference, the cloning of a strong candidate for the FRAXE gene was accomplished independently by the laboratories of Kay Davies [Chakrabarti et al., 1996; Grant Sutherland; Gecz et al., 1996] and David Nelson [Gu et al., 1996].

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